

USSN 09/656,640

Page 2.

IN THE CLAIMS

Claims 1 – 11 withdrawn.

12. (AMENDED) An isolated polynucleotide encoding an enzymatically active variant of a [precursor] phenol oxidizing enzyme, [which precursor] wherein said phenol oxidizing enzyme has at least 68% identity to the amino acid sequence as disclosed in SEQ ID NO:2, said variant comprising a sequence that differs from that of said [precursor] phenol oxidizing enzyme in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto.

13. (Original) An expression vector comprising the polynucleotide of claim 12.

14. (Original) A host cell comprising the expression vector of claim 13.

15. (Original) The host cell of claim 14, wherein said host cell is a filamentous fungus.

16. (Original) The host cell of claim 15, wherein said fungus is an Aspergillus species or a Trichoderma species.

17. Withdrawn

18. (AMENDED) A method for obtaining a phenol oxidizing enzyme variant [derived] from a Stachybotrys species, [said variant having at least one altered property relative to a precursor phenol oxidizing enzyme, which comprises] comprising the steps of:

mutagenizing a gene encoding [said precursor] a phenol oxidizing enzyme, which [precursor] phenol oxidizing enzyme comprises an amino acid sequence having at least 68% identity to the amino acid sequence shown in SEQ ID NO:2;

USSN 09/656,640

Page 3

introducing the mutant gene into a host strain whereby a transformed host strain is obtained;

growing said transformed host whereby said mutant gene is expressed and a phenol oxidizing enzyme variant, differing from said [precursor] phenol oxidizing enzyme by one or more amino acid substitutions, is identified by recovering said variant and screening it for increased phenol oxidizing activity and/or increased pH optimum.

19. (Original) The method of claim 18, wherein said one or more amino acid substitutions correspond to amino acid positions selected from the group consisting of 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573 of said SEQ ID NO:2 sequence.

20. (Original) The method of claim 18, wherein said mutagenized gene is a cloned Stachybotrys gene, preferably a cloned Stachybotrys chartarum gene, or a cloned gene capable of hybridizing to such a Stachybotrys gene under conditions of intermediate to high stringency.

21. (AMENDED) A method for producing a variant of a [precursor] phenol oxidizing enzyme, which [precursor] enzyme comprises an amino acid sequence having at least 68% identity to the amino acid sequence shown in SEQ ID NO:2; said method comprising the steps of:

a) culturing a host cell comprising a polynucleotide encoding said variant, wherein said variant differs from said [precursor] phenol oxidizing enzyme sequence in at least one of the positions 48, 67, 70, 76, 83, 98, 115, 119, 134, 171, 175, 177, 179, 188, 236, 246, 253, 254, 269, 272, 296, 302, 308, 318, 329, 331, 346, 348, 349, 365, 390, 391, 394, 404, 415, 423, 425, 428, 434, 465, 479, 481, 483, 499, 550, 562, 570, and 573, or sequence positions corresponding thereto, under conditions suitable for the production of said variant; and

(b) optionally recovering said variant produced.

USSN 09/656,640

Page 4

Claims 22-24 withdrawn.

25. (NEW) The isolated polynucleotide of claim 12, wherein said polynucleotide encodes a variant which comprises a sequence that differs from that of the phenol oxidizing enzyme in at least one of the positions 254, 272, 346, 348, 394, and 425, or sequence positions corresponding thereto.

26. (NEW) The isolated polynucleotide of claim 12 wherein said polynucleotide encodes an amino acid substitution at one or more of the positions or position sets: 76/254/302; 76/254/302/188; 76/254/302/394/425; 119/254/329; 119/254/390; 119/254/415; 171/254/346; 236/254; 254; 254/272; 254/302/346/348; 254/346/348; 254/394; 254/550; and 394/425.

USSN 09/656,640

Page 5

27. (NEW) The isolated polynucleotide of claim 12 encoding a variant having at least one amino acid substitution or substitution set selected from:

|                              |
|------------------------------|
| N391S                        |
| G115S                        |
| D562G                        |
| D394N/V425M                  |
| V134I/H177Y                  |
| L499F                        |
| M254F                        |
| M254F/L499F                  |
| M98L/M254F                   |
| L76W/M254F                   |
| M254F/F349Y                  |
| H175V                        |
| H177V                        |
| L76W/M254F/E302V             |
| M254F/D394N/V425M            |
| L76W/M254F/E302V/D394N/V425M |
| M254F/A296S                  |
| M254F/W318Y                  |
| M254F/L48Y                   |
| M254F/R83K                   |
| M254F/M188F                  |
| M254F/Q246H                  |
| M254F/S331T                  |
| M254F/V483T                  |
| M254F/R67T                   |
| V119L/M254F/N70V             |
| M254F/N70V                   |
| M254F/D308S                  |
| M254F/E365T                  |
| M254F/S415A                  |
| M254F/R423A                  |
| M254F/D428G                  |
| M254F/R434E                  |
| M254F/E465M                  |
| M254F/A479G                  |
| M254F/N550A                  |
| P253A                        |
| V119L/M254F/A269M            |
| M254F/A269M                  |
| V119L/M254F/G329N            |
| M254F/G329N                  |
| M254F/S331A                  |

USSN 09/656,640

Page 6

|                         |
|-------------------------|
| M254F/E346V/E348Q       |
| V119L/M254F/E346V       |
| M254F/E346V             |
| V119L/M254F/A390P       |
| M254F/A390P             |
| M254F/N404T             |
| V119L/M254F/S415L       |
| M254F/S415L             |
| M254F/R481G             |
| M254F/A573N             |
| M254F/A573N/F570L       |
| M254F/L76W/E302V/M188K  |
| M254N                   |
| M254L                   |
| M254A                   |
| M254I                   |
| M254E                   |
| M254S                   |
| M254H                   |
| M254V                   |
| M254T                   |
| M254P                   |
| M254G                   |
| M254K                   |
| M254C                   |
| M254F/D394G             |
| M254F/D394V             |
| M254F/D394S             |
| M254F/D394H             |
| M254F/D394P             |
| M254F/D394Y             |
| M254F/D394W             |
| M254F/D394N             |
| M254F/M179F             |
| M254F/M179V             |
| M254F/M179P             |
| M254F/M179G             |
| M254F/M179E             |
| M254F/M179L             |
| M254F/I181D             |
| M254F/S180F/I181L       |
| M254F/E346V/E302I       |
| M254F/E346V/E302K       |
| M254F/E346V/E348Q/E302F |

USSN 09/656,640

Page 7

|   |
|---|
| M254F/E346V/E348Q/E302A                   |
| M254F/E346V/E348Q/E302L                   |
| M254F/E346V/E302C                         |
| M254F/E346V/E302V                         |
| M254F/E346V/M171T                         |
| M254F/E346V/E348Q/M171P                   |
| M254F/E346V/E348Q/M171L                   |
| M254F/E346V/M171Y                         |
| M254F/E346V/E348Q/M171V                   |
| M254F/E346V/M171S                         |
| M254F/E346V/M171R                         |
| M254F/E346V/E348Q/M171F                   |
| M254F/E346V/M171K                         |
| M254F/E346V/E348Q/M171Q                   |
| M254F/E346V/E348Q/M171N                   |
| M254F/E346V/E348Q/M171N/L172H             |
| M254F/S272L                               |
| M254F/E236K                               |
| M254F/E346V/E348Q/M188K/D394W/S272L/E236K |
| M254F/E346V/E348Q/M188K/D394W/E236Q       |
| M254F/E346V/E348Q/M188K/D394W/E236K       |
| M254F/E346V/E348Q/M188K/D394W/E236D       |
| M254F/E346V/E348Q/M188K/D394W/E236A       |
| M188K/M254F/E346V/E348Q/D394W             |

28. (NEW) The isolated polynucleotide of any one of claims 12, 25, 26, or 27, wherein said phenol oxidizing enzyme has at least 80% identity, and preferably at least 85% identity, to the amino acid sequence disclosed in SEQ ID NO:2.

29. (NEW) The isolated polynucleotide of any one of claims 12, 25, 26, or 27, wherein the phenol oxidizing enzyme has at least 90% identity, and preferably at least 95% identity, to the amino acid sequence disclosed in SEQ ID NO:2.

30. (NEW) The isolated polynucleotide of claim 29, wherein the phenol oxidizing enzyme has the amino acid sequence disclosed in SEQ ID NO:2.

31. (NEW) The isolated polynucleotide of claim 12, wherein the phenol oxidizing enzyme variant has increased phenol oxidizing activity at high pH.

USSN 09/656,640

Page 8

32. (NEW) The isolated polynucleotide of claim 31, wherein the variant has a pH optimum of at least 8, and preferably at least 9.

33. (NEW) The isolated polynucleotide of claim 12, wherein said phenol oxidizing enzyme is obtainable from a *Stachybotrys* species, preferably *Stachybotrys chartarum*.

Respectfully submitted,



H. Thomas Anderton, Jr.  
Registration No. 40,895

Date: January 22, 2004

Genencor International, Inc.  
925 Page Mill Road  
Palo Alto, CA 94304-1013  
Tel: 650-846-7544  
Fax: 650-845-6504